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EXAMINER	
SODERQUIST, ARLEN	
ART UNIT	**PAPER NUMBER**
1743	

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Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	10/048,244	BLUMENTHAL, DONALD K.	
	Examiner	Art Unit	
	Arlen Soderquist	1743	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *20 September 2004*.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-27* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-27* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

1. The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

2. Claims 1-9, 13, 15 and 21 are rejected under 35 U.S.C. 102(b) as being anticipated by Packard (*J. Phys. Chem. B* **1997,** *101*, 5070-5074, hereinafter referred to as Packard '97 or *J. Phys. Chem. B* **1998,** *102*, 752-758, hereinafter referred to as Packard '98 both newly cited and applied).

In the paper Packard '97 discusses structural characteristics of fluorophores that form intramolecular H-type dimers in a protease substrate. They designed and synthesized a new class of profluorescent protease substrates whose spectral properties fit the exciton model; more specifically, spectra of these polypeptides which were doubly labeled with rhodamines showed blue-shifted absorption peaks and fluorescence quenching, both indicators of H-dimer formation. In the work described here NorFES, an undecapeptide which is cleaved by the serine protease elastase, was homodoubly labeled on opposite sides of its cleavage site with six fluorophores in order to identify structural elements of dyes which influence intramolecular H-type dimer formation. Absorption and fluorescence spectra of these six substrates obtained before and after enzymatic cleavage indicate that the exciton band is strongest in the peptide doubly labeled with tetramethylrhodamine, followed by rhodamine-X, and then (diethylamino)coumarin. In contrast, spectra of NorFES homodoubly labeled with fluorescein, hydroxycoumarin, or pyrene do not exhibit exciton bands. These data suggest that factors significant in H-type dimerization are as follows (in decreasing order): delocalized charge, symmetry, and magnitude of the lowest energy electronic transition dipole. Surprisingly, in the group of fluorophores in this study, no evidence for hydrophobic interactions as an important influence was observed.

In the paper Packard '98 discusses intramolecular resonance dipole-dipole interactions in a profluorescent protease substrate. In this study NorFES, an undecapeptide containing an amino acid sequence recognized by the serine protease elastase, was covalently labeled with two xanthenes, one on each side of its cleavage site, to serve as a tool for examination of intramolecular resonance dipole-dipole interactions. To this end using all possible combinations

from the group of xanthenes including fluorescein, tetramethylrhodamine, and rhodamine-X, three heterobichromophoric and three homobichromophoric NorFES derivatives were synthesized; their absorption and fluorescence spectra were measured both before and after cleavage by elastase. In the heterobichromophoric substrates the fluorescence of the fluorophore that would be the nominal donor in a Förster model system was quenched. Since the fluorescence intensity of the nominal acceptor in these substrates was also decreased, these data were not consistent with the Förster model. Rather, spectra for all six doubly labeled peptides could be explained by delocalization of excitation over each substrate's two fluorophores. Thus, by taking into account dipole-dipole interactions between two dyes placed in close proximity to each other, the spectral properties observed could not be ascribed to the monomeric components but were the unique optical signature of each ground-state dimer. The first paragraph of the introduction is relevant to the claims and is reproduced below with added emphasis and removal of reference citations from the body of the text.

> " Pairs of xanthene dyes, e.g., fluoresceins and rhodamines, have been used for defining distances within and between macromolecules for many years. **The interpretation most often applied to these spectral data is based on the Förster model of resonance energy transfer**. *Correct quantitation in the application of Förster's theory is dependent on accurate evaluation of parameters* such as *the donor's fluorescence lifetime and quantum yield in the absence of the acceptor*, **the overlap integral between donor and acceptor,** and κ^2, *the orientation factor between the two chromophores*. While most discord revolves around the methodology used in calculating the latter, other aspects of applying the Förster equation to spectral data can also be tenuous. For example **NorFES is an undecapeptide which we recently synthesized and derivatized with two different rhodamines believed to be a good Förster pair; however, by comparing the spectral data of this heterodoubly labeled substrate with those of relevant singly as well as homodoubly labeled substrates we found the intramolecular dipole-dipole interaction between the two dyes to be *well-described not by Förster but by exciton theory*."**

Thus, a substrate that was designed to be the type that would be of the Förster or fluorescence resonance energy transfer type was actually work through a non-fluorescence resonance energy transfer mechanism.

3. Claims 1-9, 13, 15 and 21 are rejected under 35 U.S.C. 102(b) as being anticipated by Lee in view of Packard '98 as described above or de Silva (newly cited and applied). In the paper Lee teaches a new approach to assay endo-type carbohydrases using bifluorescent-labeled substrates for glycoamidases and ceramide glycanases. Glycoamidases and ceramide glycanases

are important "endo-type" enzymes for structural elucidations of glycoconjugates as well! as for construction of neoglycoconjugates. The assay methods currently available for these enzymes are tedious and do not permit continual assay of the enzyme activities. The authors modified a desialylated biantennary glycopeptide with 2-naphthylacetic acid at the N-terminus and at the nonreducing terminal galactosyl residues with mono-N-dansylethylenediamine, via a specific oxidation of the C-6 hydroxyl group with galactose oxidase. See figure 1 for the two substrates used. In such a substrate, the naphthyl fluorescence (λ_{em} = 355 nm) is quenched due to absorption of its emitted light by the dansyl group, which in turn results in emission of fluorescence (λ_{ex} = 520 nm) by the latter. However, when the link between the two fluorophores is severed (a covalent modification) by glycoamidase (PNGase), the energy transfer ceases to occur. Consequently the emission of the dansyl fluorescence and the quenching of naphthyl fluorescence diminish or disappear. Likewise the energy transfer between the fluorophores in an alkyl lactoside containing a dansyl group at the terminal position of aglycon and a 2-naphthylmethyl group on the galactosyl residue is also eliminated by the glycosidic cleavage by a ceramide glycanase from American leech, Macrobdella decora, resulting in enhancement of the naphthyl emission and decrease in the dansyl emission. The substrates presented here permit continuous fluorescent monitoring of the enzymic reaction. This allows precise analyses of enzyme kinetics not possible with the conventional assay methods for the endo-type enzymes which usually require separation of reaction products. The Lee reference does not teach that the quenching is due to a non-fluorescence resonance energy transfer mechanism.

In the review paper de Silva discusses signal recognition events with fluorescent sensors and switches. Information relevant to the instant claims is found in the discussion of the section on electronic energy transfer. This section deals with bifluoric systems where the photoactive units are nonidentical. A couple of sections from this part of the review paper are reproduced below with added emphasis and removal of reference citations from the body of the text. The first section start in about the middle of the paragraph bridging pages 1555-1556 and includes the first part of the following paragraph.

> " Mechanistic dilemmas are common because **two or possibly three modes of EET are available to the larger supermolecules**. *Electron exchange, dipole-dipole coupling* and **emission-reabsorption** *are considered to be important at short-, medium-, and long-range*

respectively. **As usual, the borders are ill defined, and the fact that many supramolecular dimensions are in this borderland adds to the uncertainty. The situation is not helped by the fact that the electron exchange model contains unquantifiable parameters. The effects of orbital penetration were not realized until recently.** *EET is of course essential to the efficient functioning of many signaling systems based on MC (section V) and triplet (section VII) excited states.*"

" **As the following pages will demonstrate, almost all the fluorescent EET-based signaling systems for analytes depend on conformational changes caused by the recognition process. In other words, the distance dependence of EET is being exploited. The other crucial controller of EET, the spectral overlap integral between donor emission and acceptor absorption, is hardly made use of.** "

This section shows that there are a number of possible mechanisms that come to play in larger bifluoric systems having different photoactive groups. The next section is the first two paragraphs of the right column of page 1557.

" Fluorescein and rhodamine-based fluorophores have been perennial favorites in biological assays. However, an exploitation of a strong interaction between the pair is rare. **Herron and co-workers** use this approach to develop a homogeneous immunoassay method. These two fluorophores are attached to the N- and C- terminals of an oligopeptide which is recognized by an antibody raised against the important analyte hCG (human chorionic gonadotrophin). It is particularly notable that the double labeling of the relatively small oligopeptide is far easier to perform than on the large biomolecule. **The flexibility of the oligopeptide linker means that the terminal dyes πstack together to destroy each other's fluorescence.** *Antibody binding to the central oligopeptide straightens it sufficiently to force the two fluorophores apart to a safe distance. Normal EET takes over and strong emission is seen from the rhodamine acceptor.* The emission from the fluorescein donor remains weak whether the antibody is present or not i.e. ratioing of the two emission bands is feasible. Homogeneous DNA assays (section V) should also benefit from this general approach. "

" *The spotlight remains on the oligopeptide linkers betwen EET pairs of photoactive components as we near the end of our journey along the recognition-fluorescence interface.* In the present *example developed by Krafft for protease assays, the C-terminal carries a 2-aminonaphthalene-5-sulfonate fluorophore as EET donor and the N-terminal is elaborated into 4-[[4'-(dimethylamino)phenyl]azo]benzamide as the acceptor. This EET pair maintains considerable efficiency even across a decapeptide without special folding, i.e., the donor's fluorescence is substantially quenched.* **Application of Herron's approach with π-stacking fluorophore pairs should allow the use of longer oligopeptides in selective protease assays if that becomes necessary. Hydrolysis of the peptide linker by the protease switches "on" the fluorescence since the EET acceptor has been disconnected.** *Rapid screening of protease inhibitors with such irreversible signaling systems could hold the key to therapies for disease such as AIDS and Alzheimer's.* "

This section gives specific examples of EET with biological systems including the interaction in which π-stacking is involved in the fluorescence quenching mechanism.

From the teachings of Packard '98 and de Silva it is clear that multiple mechanisms are known to be available in a system as described by Lee and the description of one of these mechanisms as the mechanism present does not exclude other mechanisms from being present or the actual mechanism that is causing the fluorescence quenching in one of the possible conformations of the Lee substrate.

4. Claims 1, 3, 5, 13, 15 and 21 are rejected under 35 U.S.C. 102(b) as being anticipated by Zhang in view of Packard '98 or de Silva as described above. In the paper Zhang presents a fluorogenic substrate for measuring α-amylase (EC 3.2.1.1) activity that was prepared by double-labeling soluble starch with 5-(4,6-dichlorotriazin-2-yl)aminofluorescein and Procion Red MX 8B. Because the absorption spectrum of Procion Red MX 8B overlaps the fluorescein emission spectrum, Procion Red efficiently quenches fluorescein emission when it is closer than the critical radius for fluorescence energy transfer. When amylase catalyzes the cleavage of a starch molecule between a fluorescein and a Procion Red MX 8B, the distance between the two labels increases and the degree of quenching decreases. The rate at which the fluorescence intensity increases is proportional to amylase activity. To maximize the sensitivity it is critical to maximize the amount of Procion Red MX 8B coupled to the starch and to use a high-precision spectrofluorometer which can measure a small rate of increase in fluorescence above a large constant background. The Zhang reference does not teach that the quenching is due to a non-fluorescence resonance energy transfer mechanism.

From the teachings of Packard '98 and de Silva it is clear that multiple mechanisms are known to be available in a system as described by Zhang and the description of one of these mechanisms as the mechanism present does not exclude other mechanisms from being present or the actual mechanism that is causing the fluorescence quenching in one of the possible conformations of the Zhang substrate.

5. Claims 1-5, 7-9, 13, 15 and 21 are rejected under 35 U.S.C. 102(b) as being anticipated by Meldal in view of Packard '98 or de Silva as described above. In the paper Meldal teaches anthranilamide and nitrotyrosine as a donor-acceptor pair in internally quenched fluorescent substrates for endopeptidases and multicolumn peptide synthesis of enzyme substrates for

subtilisin Carlsberg and pepsin. The preparations of Nα-Fmoc-3-nitro-L-tyrosine and N-Boc-anthranilic acid Dhbt ester (Fmoc = fluoren-9-ylmethyloxycarbonyl; Boc = tert-butyloxycarbonyl; Dhbt = 3,4-dihydro-4-oxo-1,2,3-benzotriazo-3-yl) and their application to parallel multiple column solid-phase peptide synthesis is described. A series of peptide substrates containing an anthraniloyl group at the N-terminus and a 3-nitrotyrosyl residue close to the C-terminus were synthesized. The fluorescence of the anthraniloyl group, intramolecularly quenched by the 3-nitrotyrosine, increased with cleavage of peptide bonds situated between the two groups. The quenching mechanism was of the long-range resonance energy transfer type and long peptide substrates were constructed and used for kinetic measurements of subtilisin Carlsberg and pepsin. Complete quenching was observed even with >20 Å between the centers of the chromophores, and substrates with ≤50 Å between the chromophores were synthesized. The importance of long substrates for optimal enzymic activity was demonstrated. The Meldal reference does not teach that the quenching is due to a non-fluorescence resonance energy transfer mechanism.

From the teachings of Packard '98 and de Silva it is clear that multiple mechanisms are known to be available in a system as described by Meldal and the description of one of these mechanisms as the mechanism present does not exclude other mechanisms from being present or the actual mechanism that is causing the fluorescence quenching in one of the possible conformations of the Meldal substrate.

6. Claims 1, 3, 5, 7, 13, 15 and 21 are rejected under 35 U.S.C. 102(b) as being anticipated by Taliani in view of Packard '98 or de Silva as described above. In the paper Taliani presents a continuous assay of hepatitis C virus protease based on resonance energy transfer depsipeptide substrates. Hepatitis C virus (HCV) is the major causative agent of non-A non-B hepatitis, an important health problem with an estimated 50 million people infected worldwide. Among the possible targets for the therapeutic intervention, the serine protease contained within the N-terminal region of nonstructural protein 3 (NS3 protease) is so far the best characterized. In vitro characterization of synthetic substrates based on all the natural cleavage sites (as well as a series of analogs) has consistently revealed poor kinetic parameters, making them unsuitable for sensitive high-throughput screening. To overcome these difficulties, they recently developed depsipeptide substrates incorporating an ester bond between residues P1 and P'1. Due to ready

transesterification of the scissile bond to the acyl-enzyme intermediate, these substrates showed very high k_{cat}/K_m values, enabling detection of activity with subnanomolar NS3 concentrations. They used the same principle to synthesize internally quenched depsipeptide fluorogenic substrates based on resonance energy transfer between the donor/acceptor couple 5-[(2'-aminoethyl)amino]naphthalene sulfonic acid/4-[[4'-(dimethylamino)phenyl]azo]benzoic acid, and developed a continuous assay for NS3 activity. Substrate cleavage is linear with enzyme concentration; depending on the conditions chosen, they estimated a detection limit for NS3 between 1 nM and 250 pM. The suitability of the assay for evaluation of inhibitors was established using as competitor a tridecapeptide corresponding to the natural NS4A/4B cleavage site; this gave an IC50 of 30 μM, well in agreement with the previously found K_m value (40 μM). The Taliani reference does not teach that the quenching is due to a non-fluorescence resonance energy transfer mechanism.

From the teachings of Packard '98 and de Silva it is clear that multiple mechanisms are known to be available in a system as described by Taliani and the description of one of these mechanisms as the mechanism present does not exclude other mechanisms from being present or the actual mechanism that is causing the fluorescence quenching in one of the possible conformations of the Taliani substrate.

7. Claims 1, 3-5, 7, 12-13, 15 and 21 are rejected under 35 U.S.C. 102(b) as being anticipated by Zandonella in view of Packard '98 or de Silva as described above. In the paper Zandonella teaches fluorogenic alkyldiacyl glycerols as substrates for the determination of lipase activity and stereoselectivity. They synthesized enantiomeric alkyl-diacyl glycerols containing pyrene as a fluorophore, and the trinitrophenylamino residue as a fluorescence quencher, both covalently bound to the ω-end of the respective acyl chains. Fluorescence is efficiently quenched due to resonance energy transfer in the intact molecules. Chemical or enzymic release of the fatty acyl chains lead to fluorescence dequenching. From the time-dependent increase in fluorescence intensity lipase activity and stereoselectivity can be determined, if enantiomerically pure substrates are used. The Zandonella reference does not teach that the quenching is due to a non-fluorescence resonance energy transfer mechanism.

From the teachings of Packard '98 and de Silva it is clear that multiple mechanisms are known to be available in a system as described by Zandonella and the description of one of these mechanisms as the mechanism present does not exclude other mechanisms from being present or the actual mechanism that is causing the fluorescence quenching in one of the possible conformations of the Zandonella substrate.

8. Claims 1-7, 9, 11, 13, 15 and 21 are rejected under 35 U.S.C. 102(b) as being anticipated by Hirano (JP 11-56398) in view of Packard '98 or de Silva as described above. In the published application Hirano teaches determination of double-stranded nucleic acids-cleaving enzyme activities by fluorescence resonance energy transfer (FRET) analysis. In the method to determine the double-stranded nucleic acid enzyme activity, a nucleic acid capable of forming an intermolecular duplex (a single-stranded probe having, in the molecule, a recognition base sequence portion for a double-stranded nucleic acid splitting enzyme and a base sequence portion complementary to the above base sequence portion) is prepared and used as a substrate. The nucleic acid is labeled with an energy donor (e.g. fluorescein) and an energy acceptor (e.g. rhodamine X) at both ends, respectively. The recognition base sequence portion is hybridized with the base sequence portion complementary thereto to form a hybrid and reacted with the enzyme. The increase of fluorescence resulting from the enzymic digestion of the nucleic acid substrate can be observed by fluorometry. The method was demonstrated by digestion of substrates for restriction endonucleases HindIII and PvuII. The Hirano reference does not teach that the quenching is due to a non-fluorescence resonance energy transfer mechanism.

From the teachings of Packard '98 and de Silva it is clear that multiple mechanisms are known to be available in a system as described by Hirano and the description of one of these mechanisms as the mechanism present does not exclude other mechanisms from being present or the actual mechanism that is causing the fluorescence quenching in one of the possible conformations of the Hirano substrate.

9. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

The factual inquiries set forth in *Graham* **v.** *John Deere Co.*, 383 U.S. 1, 148 USPQ 459 (1966), that are applied for establishing a background for determining obviousness under 35 U.S.C. 103(a) are summarized as follows:

1. Determining the scope and contents of the prior art.
2. Ascertaining the differences between the prior art and the claims at issue.
3. Resolving the level of ordinary skill in the pertinent art.
4. Considering objective evidence present in the application indicating obviousness or nonobviousness.

10. Claims 1-27 are rejected under 35 U.S.C. 103(a) as being unpatentable over Macala or Schultz (US 5,580,747) or Ventura in view of Blumenthal, Mathies (US 5,654,419) and Hirano, Lee, Meldal, Taliani, Zandonella, or Zhang and Packard '98 or de Silva (last eight as explained above).

In the paper Macala teaches measurement of cAMP-dependent protein kinase activity using a fluorescent-labeled Kemptide. Traditional protein kinase assays include the use of ^{32}P-labeled ATP as phosphate donor and a substrate protein or peptide as phosphoreceptor. Since this approach has a number of drawbacks in addition to generating ionizing radiation, several non-isotopic methods have been developed. Although shown to reflect the activity of purified enzymes, none have been demonstrated to detect physiological changes in endogenous enzyme activity in cell homogenates. Studies were performed to examine the kinetics, reproducibility, and optimal assay conditions of a novel non-radioisotopic kinase assay that detects protein kinase A (PKA) activity by phosphorylation of the peptide substrate, Kemptide, covalently bound to a fluorescent molecule (fluorescamine-labeled Kemptide; f-Kemptide). Fluorescence was determined by spectrofluorometry with excitation at 568 nm and emission at 592 nm. Basal and agonist-induced PKA activities in epithelial cell homogenates were measured. The kinetics of f-Kemptide were similar to the standard radioisotopic method with intra-assay and inter-assay variations of 5.6 ± 0.8% and 14.3 ± 2.6%, respectively. Neither fluorescence quenching nor enhancing effects were found with consistent amounts of homogenate protein. Specific PKA activity was determined as the IP20-inhibitable fraction to account for nonspecific phosphorylation, perhaps due to S6 kinase or a similar enzyme. The basal activity of 38% of total PKA in A6 cells increased by 84% after exposure to vasopressin and by 58% after short exposure to forskolin. In T84 cells exposed to VIP there was a 360% increase over basal

activity. These results show that f-Kemptide exhibits acceptable kinetics, and that the assay system can quantitatively and reproducibly measure basal and stimulated PKA activity in cell homogenates. Macala does not teach the substrate having two dyes attached or a library of compounds.

In the patent Shultz teaches a non-radioactive assay and purification of proteins, and particularly to the non-radioactive assay and purification of protein kinases, phosphatases and protease by incubating the enzyme with a substrate modified peptide to form a product modified peptide under conditions where the enzyme is active. The product modified peptide and substrate modified peptide are then separated, and the product modified peptide is measured. The present invention is also directed to kits and bioreagents for performing the assays. Table 1 of the patent shows a list of substrates that have a fluorescent dye attached to the substrate. Shultz does not teach the substrate having two dyes attached or a library of compounds.

In the paper Ventura teaches phorbol ester regulation of opioid peptide gene expression in myocardial cells and the role of nuclear protein kinase C. Opioid peptide gene expression was characterized in adult rat ventricular cardiac myocytes that had been cultured in the absence or the presence of phorbol 12-myristate 13-acetate. The phorbol ester induced a concentration- and time-dependent increase of prodynorphin mRNA, the maximal effect being reached after 4 hours of treatment. The increase in mRNA expression was suppressed by incubation of cardiomyocytes with staurosporine, a putative protein kinase C inhibitor, and was not observed when the cells were cultured in the presence of the inactive phorbol ester 4a-phorbol 12,13-didecanoate. Incubation of cardiac myocytes with phorbol 12-myristate 13-acetate also elicited a specific and staurosporine-sensitive increase in immunoreactive dynorphin B, a biologically active end product of the precursor, both in the myocardial cells and in the culture medium. In vitro run-off transcription assays indicated that transcription of the prodynorphin gene was increased both in nuclei isolated from phorbol ester-treated myocytes and in nuclei isolated from control cells and then exposed to phorbol 12-myristate 13-acetate. No transcriptional effect was observed when cardiac myocytes or isolated nuclei where exposed to 4a-phorbol 12,13-didecanoate. The phorbol ester-induced increase in prodynorphin gene transcription was prevented by pretreatment of myocytes or isolated nuclei with staurosporine, suggesting that myocardial opioid gene expression may be regulated by nuclear protein kinase C. In this regard,

cardiac myocytes expressed protein kinase C-α, -δ, -ε, and -ζ, as shown by immunoblotting. Only protein kinase C-δ and protein kinase C-ε were expressed in nuclei that have been isolated from control myocytes, suggesting that these 2 isotypes of the enzyme may be part of the signal transduction pathway involved in the effect elicited by the phorbol ester an opioid gene transcription in isolated nuclei. The incubation of myocardial nuclei isolated from control cells in the presence of a protein kinase C activator induced the phosphorylation of the myristylated alanine-rich protein kinase C substrate peptide, a specific fluorescent substrate of the enzyme. The possibility that prodynorphin gene expression may control the heart function through autocrine or paracrine mechanisms is discussed. Ventura does not teach the substrate having two dyes attached or a library of compounds.

In the paper Blumenthal reviews the development and characterization of fluorescently-labeled myosin light chain kinase calmodulin-binding domain peptides. The development and characterization of peptides based on the sequence of the calmodulin-binding domain of skeletal muscle myosin light-chain kinase which were labeled with the fluorescent reagent, acrylodan are described. The use of these fluorescently-labeled peptides to study various aspects of calmodulin-peptide interactions including binding affinity, stoichiometry, specificity, changes in peptide conformation, and thermal stability of the peptide-calmodulin complex is demonstrated. Page 46 discusses the preparation of analogs by replacing different amino acids within a natural peptide sequence to examine these properties. Blumenthal also teaches the formation of a library of peptides with different fluorescent labels. Page 46 also discusses the change in the acrylodan fluorophore as the environment changes and how this is useful in determining various properties. The peptides exhibit many of the salient features seen with calmodulin-target enzyme interactions. The fluorescently-labeled peptides should serve as useful models for studying calmodulin-target enzyme interactions at the molecular level.

In the patent Mathies discusses fluorescent labels and their use in separations. Probes labeled with energy transfer coupled dyes for use in hybridization and DNA sequencing. Sets of fluorescent labels carrying pairs of donor and acceptor dye molecules bonded to a polymeric backbone in an energy transfer relationship and method of their analytical use are described. These labels are designed for efficient excitation of the donors at a single wavelength and

emission from the acceptor in each of the pairs at different wavelengths. The different molecules having different donor-acceptor pairs can be modified to have substantially the same mobility under separation conditions, by varying the distance between the donor and acceptor in a given pair. Particularly, the fluorescent compositions find use as labels in sequencing nucleic acids. Of particular interest are the labels where the polymeric backbone is a nucleic acid and the donor fluorophore is bonded to the 5' terminus of the nucleic acid. These labels find use as primers in applications involving nucleic acid chain extension, such as sequencing, PCR and the like. Sequencing primers labeled with two fluorophores were constructed and their fluorescent properties optimized. Dual fluorophore-labeled PCR primers for sizing of short tandem repeats (STRs) in the tyrosine hydroxylase, thrombopoietin, cytostatic factor and von Willebrand factor genes were also prepared. These primers were used to amplify the STRs and the amplicons were separated by capillary gel electrophoresis. Column 4 gives many examples of the types of dyes that may be used as the donor and acceptor pairs.

It would have been obvious to one of skill in the art at the time of the invention to incorporate a double label selected from those taught by Mathies, Hirano, Lee, Meldal, Packard '98, de Silva, Taliani, Zandonella or Zhang in the Macala, Shultz or Ventura substrates because of the ability to detect changes in the substrate due to covalent modifications of the substrates as shown by Hirano, Lee, Meldal, Packard '98, de Silva, Taliani, Zandonella or Zhang and the ability to carry out a continuous monitor without separation and from the teachings of Packard '98 and de Silva it is clear that multiple mechanisms are known to be available in a system as described by Macala, Shultz or Ventura and the description of one of these mechanisms as the mechanism present does not exclude other mechanisms from being present or the actual mechanism that is causing the fluorescence quenching in one of the possible conformations of the substrate. One of skill in the art would also have recognized that libraries of substrates as taught by Blumenthal would have allowed the Macala, Shultz or Ventura substrates to be used for characterizing enzyme properties as shown by Blumenthal.

11. Applicant's arguments with respect to the claims have been considered but are moot in view of the new ground(s) of rejection. From the teachings of Packard '98 and de Silva it is clear that multiple mechanisms are known to be available in a system as claimed and described by the applied references and the description of one of these mechanisms as the mechanism

present does not exclude other mechanisms from being present or the actual mechanism that is causing the fluorescence quenching in one of the possible conformations of the substrate.

12. Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL**. See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

13. The prior art made of record and not relied upon is considered pertinent to applicant's disclosure. The additionally cited art relates to fluorescently labeled substrates and their analytical use.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Arlen Soderquist whose current telephone number is (571) 272-1265 as a result of the examiner moving to the new USPTO location. The examiner's schedule is variable between the hours of about 5:30 AM to about 5:00 PM on Monday through Thursday and alternate Fridays.

A general phone number for the organization to which this application is assigned is (571) 272-1700. The fax phone number to file official papers for this application or proceeding is (703) 872-9306.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

Arlen Soderquist

December 1, 2004

ARLEN SODERQUIST
PRIMARY EXAMINER